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BIOLOGICAL BULLETIN

RETINAL REFLEXES OF NARCOTIZED ANIMALS TO SUDDEN CHANGES OF INTENSITY OF ILLUMINATION.

A. T. CAMERON AND C. H. O'DONOGHUE.

(From the Marine Biological Station, Nanaimo, B. C., Canada.)

In March last, Cameron and Sedziak, working in Winnipeg, published a note[1] describing a peculiar reaction of the frog (*R. pipiens* from Illinois), narcotized with benzene, to sudden changes of illumination, a reaction noted incidentally in rehearsing a class experiment on the relative pharmacological effects of different hydrocarbons.

"Injection of 0.5 c.c. of pure benzene into the anterior lymph sac of a frog weighing about fifty grams produces distinct effects within a few minutes. The eyes close, movements become sluggish, when turned over the animal recovers its normal position with difficulty, and in most cases can no longer do so after ten minutes. The body often becomes characteristically arched. The usual reflexes gradually disappear. Breathing ceases. With the gradual disappearance of the conjunctival reflex the eye opens, the nictitating membrane is usually drawn down, and the 'retinal' reflex can be observed. Initially it would appear to be evoked by any rapid change in intensity of illumination. When the hand is passed suddenly between the frog's eye and the source of light, within one or two seconds the head is moved, and usually the hind limbs also. When a bright light (electric light at a distance of one foot) is suddenly switched on, the response follows. On switching the light off the response is more marked. Direction of light to and from the pupil only, the rest of the head being screened from light changes, evokes the response. Gradually the response to sudden increase of illumination ceases. The response to sudden decrease

of illumination persists much longer. Cessation of stimulus therefore appears to be more powerful than application of stimulus. Ether anæsthesia abolishes the response to increase of illumination first.

"The maximum effect is reached at from twenty to twenty-five minutes after injection, and the reflex disappears in another twenty to sixty minutes, or occasionally after even a longer interval. Quality of light does not affect the result; both red and blue light evoke the reflex. Intensity of light appears to be the governing factor. A retina fatigued by the shining of a strong light upon it for some minutes does not call forth such an active response on switching off the light. There may be no movement, but after a minute or two the response can be again elicited by further light stimulation. The degree of response varies in different animals from a slight head movement to such vigorous movements of the hind limbs as would be excited by a strong electric current applied externally. After the reflex has disappeared electrical stimulation still produces active muscular responses. . . . The optimum dose of benzene appears to be 1.2 per cent. of the body weight. Two per cent. induces convulsions and death rapidly, and the retinal reflex can not be observed, while 0.7 per cent. may not produce any definite effect. . . . Similar changes have been observed with toluene, and to a less extent with phenol. Chlorbenzene and brombenzene give the reflex when injected in somewhat larger doses. The reflex could not be detected after injection of similar doses of xylene, aniline, nitrobenzene, benzyl alcohol, and pyridine."

At the same time Fröhlich and Kreidl published an account[2] of similar observations on the prawn *Palæmon squilla* F. found under stones at low tide. These observations were carried out on the island of Brioni in the Adriatic, and initially were accidentally made during experiments to determine the action of certain drugs on the heat-narcosis of these prawns. Fröhlich and Kreidl found that a similar reaction to illumination changes develops in these animals when immersed in sea-water containing camphor or phenol. A small arc-light was used for illumination. Thus for camphor:

"Immersion in camphor-sea-water solution of concentration 1 : 16000 is fatal to *Palæmon* in from five to fifteen minutes. The

toxic effects appear as twitches of the limbs and claws, convulsive strokes of the tail, while, when a number of animals are placed in the same vessel, they cling to each other in an entangled mass which is not easily separable. The convulsions are of such a nature that the animal clings fast to the nearest object, especially to the soft legs of its neighbors. If at this stage of camphor poisoning before permanent paralysis sets in the animal is removed rapidly to sea-water, the toxic effects just described—muscular twitchings and convulsions—disappear rapidly. *There remains, however, a pronounced photic over-excitability,* which is manifested by the animal making *very pronounced springs* when the light intensity is rapidly increased, *but on removal of the strong light, violent springs and tail-strokes. . . . The rise of reflex-excitability on sudden lessening of light-intensity was constant at the same phase of camphor action.* . . . There was no trace of a reflex increase for tactile stimuli, neither by shaking the vessel, nor by gently touching the head or tail."

Similar results were obtained with phenol in sea-water. A concentration of 1:2000 was rapidly fatal, one of 1:5000 less rapid in action. Removal to sea-water before complete paralysis permitted the same light reactions to be observed, especially that of removal of light. Strychnine, ammonia, and caffeine did not produce the effect.

Fröhlich and Kreidl point out that under natural conditions various animals react more strongly to shadow than to sudden increase of light intensity. Sarasin in 1887 (*Diadema setosum*)[3] and Uexküll in 1896 (*Centrospinus longispinus*)[4] noted that sea-urchins react to shadow but not to light with movements of the spines, and Hess, in 1915,[5] showed that shadow, but not light increase, produces in them rotating movements of certain flask-shaped structures. Franz (1919)[6] has shown the existence of the shadow reactions in snails. Fröhlich and Kreidl consider that the reaction is protective, and that the difference in the "cerebrally" poisoned and centrally irritable *Palæmon* is only quantitative.

In the experiments now to be described we have endeavored to link up the experiments of these workers with those of Cameron and Sedziak, since the phenomenon is obviously the same in the two series.

Experimental Results.

Experiments have been carried out with benzene, phenol, camphor, and menthol. The benzene used was Merck's "highest purity, crystallizable," the phenol and menthol were commercial preparations. Two preparations of camphor were used: one, a Japanese commercial product, M. P. 174° C.; the second, a thrice resublimed preparation with M. P. 173.5° C. The latter gave somewhat more pronounced results. The menthol and camphor solutions were made by dissolving them in about twice their weight of absolute alcohol, and adding this to sea-water. The drug was precipitated in finely divided form and saturation of the sea-water followed rapidly. Tests with the same amount of alcohol in sea-water gave completely negative results. Various strengths of solution were used. These are indicated in brackets. The changes in illumination were effected by means of an acetylene lamp, which was suddenly shone on the vessel containing the animals under observation, and suddenly screened or removed. In the following account "light" signifies a sudden marked increase of illumination, "darkness" a sudden marked decrease. The animals were contained in large beakers, and sometimes in large porcelain evaporating basins.

A large number of marine species have been tested. These were selected largely with an idea of ascertaining in how wide a distribution of species the reflex could be obtained. Our choice was to some extent restricted, however, since it was limited to the species easily available.

The experiments are arranged in the order of the species tested.

1. *Cœlenterata. Hydromedusa. Gonionemus vertens.*—A common jelly-fish found amongst the eel-grass (*Zostera marina*) near the station at low tide.

Benzene (1 : 2000) produced no effect. The animal contracted normally after 15 hours. Light variations produced no discernible effect.

Camphor (resublimed, 1 : 16000) produced no effect. The animal was quiescent after two or three hours, but still contracted when touched after 18 hours. Light variations produced no discernible effects.

2. *Echinodermata. Echinoidea. Strongylocentrotus drobachiensis.*—Sea-urchins found on the under surface of the float on the station wharf.

Benzene (1 : 2000) produced an immediate immobility, and light changes ceased to produce any movement of spines. The animal was completely narcotized in six minutes.

Camphor (resublimed, 1 : 16000) produced the same result, and was fatal in less than eleven minutes.

Menthol (half-saturated) produced quiescence in three minutes. Light changes produced no discernible results.

3*a*. *Arthropoda. Crustacea. Decapoda.*

(*A*) *Hemigrapsus nudus.*—A shore crab found plentifully between tides under rocks at the station. When specimens were placed in sea-water under the experimental conditions, "darkness" produced a sudden stoppage of movement, and an occasional "flick," very slight.

Benzene (1 : 1500) produced narcosis in one or two minutes. Before this was complete the crab reacted to "light" by general activity—movement of chelæ and legs, and sometimes of the eye-stalk. "Darkness" produced no effect.

Benzene (1 : 3000). Two animals gave a "darkness" response, definite, but not much greater than normal, after one minute. After five minutes even touching them produced no response, and after ten minutes narcosis was complete.

Phenol (1 : 2000) was fatal to these crabs in from 30 to 40 minutes. Some minutes previously to death both "light" and "darkness" produced marked reflexes.

Camphor (1 : 16000) produced no effect. The crabs were normal after 6 hours' immersion. The resublimed camphor (1 : 16000) produced in a somewhat small specimen (7 grams) a "darkness" response in 10 minutes, and in a larger one in 40 minutes. In 45 minutes both gave marked responses, which continued for some time. After 15 hours' immersion slight responses could be elicited. It was doubtful that these were greater than normal. The animals were otherwise normal at that period.

(*B*) *Epialtus productus.*—A "kelp crab," a somewhat larger species, found amongst eel-grass near the station at low tide.

Benzene (1 : 2000). A large specimen immersed in this solu-

tion became more active. After 45 minutes activity had nearly ceased. The "darkness" reflex was present, but not marked. It was shown by sudden spasmodic movements of legs and chelæ. After a further 15 minutes a "light" reaction was doubtfully present; the "darkness" reaction was marked. The latter slowly decreased. After 70 minutes' immersion the crab scarcely responded to any stimulus. It was then removed to fresh sea-water and slowly recovered.

Camphor (1:16000). A smaller specimen showed a marked "darkness" reaction seven minutes after immersion. There was a general "flick" of legs and chelæ, but no coördination. "Light" produced no effect. After nine minutes' immersion the reaction was no longer given; the animal scarcely responded to any stimulus. Transferred to fresh sea-water, it did not recover.

Menthol (saturated solution). A similarly sized small specimen showed the "darkness" reaction after 12 minutes' immersion. The "light" response became evident after 22 minutes, and after 30 minutes both "light" and "darkness" responses were distinct. The animal commenced to struggle violently, shed both chelæ and three legs, and thereafter became scarcely responsive to any stimulus and (35 minutes) was evidently moribund.

(*C*) *Cancer productus.*—A fairly large crab found on the sea-bottom near the station wharf. Specimens placed in fresh sea-water under experimental conditions gave no noticeable response to changes of illumination. "Darkness" produced very occasionally slight twitches, shown chiefly by movements of the claws.

Benzene (1:1500). A medium-sized specimen (93 grams) was immersed. In six minutes a "darkness" reaction was evident. In 18 minutes both "light" and "darkness" responses were marked. The response consisted of spasmodic contractions of the legs and claws, so that the whole crab was raised. When placed on its back and tested, all the limbs suddenly contracted. The third maxilliped shut and the abdomen was brought up tightly against the carapace. After 32 minutes "light" and "darkness" responses were still evident, but slight, and on touching the animal little response was evoked. After 38 minutes the animal was apparently dead. It was removed to fresh sea-water, but did not recover.

Phenol (1:2000) was tested on a larger specimen weighing 140 grams. The "darkness" response was first noted after five minutes. After 18 minutes the "light" response was present, and the "darkness" response was much more marked. A second immediate "light" response produced a much greater reaction. The animal was by no means narcotized at this stage, tapping and touching still yielding marked responses. After 45 minutes the "darkness" response was still noticeable, but at the end of 70 minutes scarcely any responses were obtainable. The animal was removed to fresh sea-water, but did not recover.

Camphor (1:16000) was tested on a crab weighing 96 grams. The "darkness" response was just observable after 22 minutes. After 50 minutes "light" produced a response, "darkness" a much more marked one. After 77 minutes the animal remained on its back. It reacted strongly to both "light" and "darkness." The reaction was still evident after four hours and the animal was then by no means completely narcotized. After seven and one half hours only feeble responses were given and the animal was evidently moribund.

Menthol (saturated solution) was tested on a crab weighing 75 grams. The animal became very active immediately after immersion. "Darkness" produced a slight response after three minutes. After 10 minutes the reaction was very evident, but only with distinct pauses between each test. It continued, with gradual lessening, for three hours. No definite "light" response could be elicited. The animal appeared normal throughout with the exception of this response.

(*D*) *Calianassa californiensis.*—A large burrowing sand-shrimp found at low tide at Lock Bay on Gabriola Island, about three miles from the station.

Benzene (1:1500) produced a marked effect in 20 minutes. The shrimps remained on their backs with only occasional tail-twitches. After 45 minutes' immersion they were removed to fresh sea-water. They slowly recovered. During this period "light" produced definite tail movements. "Darkness" did not produce a definite effect.

Phenol (1:2000) produced complete narcosis in 17 minutes. The animals were transferred to fresh sea-water. Neither during

narcosis nor during recovery were any "light" or "darkness" reactions observable.

Camphor (1:16000) produced no effects. After four hours' immersion the animals were quite normal.

(*E*) *Spirontocaris paludicola.*—A small shrimp found amongst the eel-grass at low tide.

Camphor (resublimed, 1:16000) produced narcosis in 12 minutes. The animals did not curl up. Transferred to sea-water, they slowly recovered. During this period a "light" reaction was doubtfully present. No "darkness" reaction was observed. A somewhat larger specimen was narcotized within one minute. During recovery after transference to fresh sea-water similar doubtful effects were obtained.

(*F*) *Hippolyte californiensis.*—Small shrimps found amongst eel-grass.

Benzene (1:1500) caused at first activity. The animals became sluggish and showed some tendency to cling together in chains (apparently a similar effect to that observed by Fröhlich and Kreidl with prawns in camphor). During this period no "light" and "darkness" responses were observed. After 20 minutes the animals were transferred to fresh sea-water. Within two or three minutes "light" produced a definite response, and "darkness" a much more marked one. These consisted of slight twitches throughout the body of the animal, jerks of antennæ and legs sometimes sufficient to move the animal its own length from its original position. Tapping or touching the animal at this period produced no effect. A second experiment with (1:2000) benzene produced almost complete narcosis in 14 minutes, and, after transference to fresh sea-water, positive "light" and "darkness" responses in four minutes.

Phenol (1:2000 and 1:4000) was fatal within one minute. Phenol (1:10000) was fatal in two or three minutes. No responses to "light" or "darkness" were observed.

Camphor (resublimed, 1:16000) produced a marked action. At first the animals gave marked springs, then curled up. During this period "light" and "darkness" reactions were absent. After four or five minutes the animals were transferred to sea-water. They immediately commenced to relax. Several gave distinct re-

sponses to "darkness" similar to those described for benzene. The reactions to "light" were less certain. Some animals were killed by five minutes' immersion.

Menthol (half-saturated solution) caused almost complete narcosis in 14 minutes, during which time there was some tendency to curl up and to cling together. Transferred to fresh sea-water, during the recovery period the animals appeared to give some "light" and "darkness" responses, though these were less certain than in benzene, and certainly less great.

3b. Arthropoda. Crustacea. Peracarida.

Amphipoda.—Camphor (1 : 16000) produced no effect during 14 minutes on some specimens of a species found on eel-grass.

Isopoda. Pentidotea wosnesenskii.—A small isopod found on eel-grass. Normal animals under the conditions of experiment showed little response to "light" and "darkness." Occasional "flicks" backward of head and antennæ occurred, but could not be related to illumination changes.

Benzene (1 : 2000) produced a definite effect in five minutes, the "darkness" response being distinct, that to "light" much less, but still observable. Tapping the vessel or the table on which it was resting produced the same sort of response. The reactions persisted for 30 minutes. They consisted of movements of the antennæ, "kicks" when the animals were on their backs, and, when on their legs, these were extended simultaneously so that the whole animal was lifted, and at the same time the opercula were "flicked." The responses gradually lessened with time. The best were obtained when the animal had been brightly illuminated for one or two minutes, and then the source of bright light suddenly removed. An immersion of from two and one half to three hours proved fatal.

Phenol (1 : 2000) gave similar "darkness" and tap responses after 20 minutes. These were not so marked as with benzene, and "light" responses were not observed.

Camphor (resublimed, 1 : 16000) produced no effect in eight hours, the animals appearing completely normal. A saturated solution produced no effect.

Menthol (half-saturated solution) produced definite "light" and "darkness" responses within 10 minutes. The animals were not

so narcotized as with benzene. The response was evident as a flick back of head and antennæ. The animals ceased to respond after 90 minutes' immersion. They were transferred to fresh sea-water and during recovery gave similar but slighter responses to "darkness."

4. *Mollusca. Nudibranchia. Hermissenda opalescens.*—A specimen was found on eel-grass.

Camphor (resublimed, 1:16000) was tested on this specimen. After an hour there was some appearance of a reaction to "light," but none to "darkness." After 11 hours the animal was still just alive.

5*a*. *Vertebrata. Pisces.*

(*A*) *Cottoid* (*sp.*).—Small fish, too young for identification, about one inch long, caught in pools near low-water mark.

Benzene (1:1500) was fatal in three minutes. Benzene (1:3000) was fatal in less than 13 minutes. No responses to illumination changes could be detected. A (1:5000) solution was fatal to some animals in seven minutes. At this period in two cases a definite reaction (flick of body) was obtained with "light," but none was observed with "darkness."

Phenol (1:2000) was fatal to two of three animals in two minutes. The third was transferred to fresh sea-water and gave one or two doubtful responses to light changes.

Camphor (resublimed, 1:16000) was without effect. The fish were normal after three and one half hours.

(*B*) *Pholis ornatus.*—Blennids caught at low tide amongst the eel-grass.

Benzene (1:3000). Two small specimens, about three inches long, were almost narcotized in three minutes. They were transferred to fresh sea-water. No reaction to "light" was observed, but several definite responses to "darkness" were obtained. After 12 minutes both animals were dead.

Phenol (1:2000). Two similarly sized specimens were immersed and became narcotized in one minute. No definite light responses were observed. The animals were transferred to fresh sea-water, but did not recover. Two similar specimens were immersed in (1:4000) phenol. Definite "light" and "darkness" responses were given within one or two minutes of immersion.

The animals ceased to respond after six minutes, were transferred to fresh sea-water, but did not recover. A large blennid, apparently completely recovered from treatment with camphor, immersed in this solution, gave almost immediately a definite response to "darkness," but none to "light." It ceased to respond after five minutes.

Camphor (resublimed, 1:16000). One large specimen, about five inches long, gave a definite "light" and a more marked "darkness" response after 10 minutes' immersion. These responses consisted of a twitch of the tail and sometimes of the whole body. Tapping the vessel containing the animal induced a similar response. That to "light" ceased first. After 15 minutes' immersion the animal appeared completely unconscious. It was transferred to fresh water and recovered. It gave no definite responses during recovery. A still larger specimen immersed in this solution gave very definite responses within three minutes, those for "darkness" being much more marked. The reflex gradually disappeared. After 13 minutes breathing had stopped, but the "darkness" response could still be elicited. After 20 minutes no responses could be obtained to any stimulus. The animal was returned to fresh sea-water and recovered. Two small specimens which had been kept several hours in the laboratory were narcotized in about 10 minutes. No illumination responses were seen.

(*C*) *Siphostoma griseolineatum*.—A species of pipe-fish about six inches long caught amongst eel-grass at low tide.

Benzene (1:3000). Two animals gave a very positive "darkness" response after eight minutes' immersion. That to "light" was less. The response was indicated by a spasmodic jerk. It could be elicited during an additional 15 minutes. Both animals gave marked responses even after tap and tactile stimuli had ceased to produce much reaction, and after breathing had stopped.

Phenol (1:4000). A specimen, previously treated with camphor, and apparently completely recovered, gave, after 10 minutes' immersion, positive "light" responses and more marked "darkness" responses. A fresh specimen immersed in a (1:5000) solution gave slight responses after 10 minutes' immersion, which were more distinct for "darkness."

Camphor (resublimed, 1:16000). A specimen gave a response

to "darkness" in eight minutes. In 12 minutes it appeared narcotized, was removed to fresh sea-water for five minutes, and then replaced in the camphor solution. Subsequently it gave very marked "darkness" responses and less marked "light" reactions, these continuing during 15 minutes. It was then transferred to fresh sea-water and slowly recovered. No definite responses could be detected during recovery.

Camphor (1:16000). A specimen, kept for two and one half hours in the laboratory, was immersed. It appeared completely narcotized within a few minutes. It was transferred to fresh sea-water, and then replaced in the camphor solution. After repeating this procedure again, it gave slight but definite "light" and "darkness" responses.

Menthol (half-saturated solution) produced in two specimens complete paralysis within a few minutes. The animals became absolutely limp, in marked contrast to the action of the other compounds used, which ultimately produced rigidity. They were transferred to fresh sea-water and slowly recovered. No illumination responses could be detected at any period of treatment. A saturated solution produced an immediate "limp" paralysis. A particularly large fish (eight inches long) immersed in a one fifth saturated solution showed no apparent effect of any kind in 45 minutes.

(*D*) *Gasterosteus williamsoni microcephalus.* — Californian stiklebacks, caught when free swimming near the station wharf. Under the conditions of experiment changes in intensity of illumination produced no effect on the normal fish.

Benzene (1:1500). Three specimens were tested. One, 24 mm. long, gave distinct positive reaction to "darkness" in four minutes. This consisted of flicks of the tail and forward movements. During constant illumination at this stage the animal was quiescent. After five and one half minutes the animal appeared moribund, and on removal to fresh sea-water it did not recover. "Light" did not produce definite responses at any period. A second animal of similar size was completely narcotized in less than two minutes. Some doubtful "darkness" responses were observed. The third, somewhat larger (33 mm.) and more active, gave "darkness" responses in from four to five minutes after

immersion. After 14 minutes the animal was motionless and had ceased to respond even to direct touch. On removal to fresh seawater it slowly recovered. During this period "darkness" frequently seemed to produce a backward and upward movement four or five times the length of the fish. This immediately ceased on switching on the light, the animal sinking.

5*b*. *Vertebrata. Amphibia. Rana pipiens.*—The results of Cameron and Sedziak for the frog under the action of benzene and phenol have already been quoted. In order to complete this record, the following experiments were carried out at the University of Manitoba:

Camphor (commercial preparation) was tested in two ways. (*a*) 0.5 c.c. of a 20 per cent. solution in absolute alcohol was injected into the anterior lymph sac of a frog weighing 60 grams. There resulted gradual onset of paralysis, the respiratory movements being the last retained. The animal died in about one and one half hours. Change of illumination produced no effect at any period. Since the immediate result of contact of the solution with body fluid is precipitation of finely divided camphor, with subsequent slow solution, no certain dosage in this experiment could be attempted. In a second frog injected with a smaller dose paralysis intervened sooner, and again no "light" and "darkness" effects could be elicited. (*b*) Two frogs were immersed in water saturated with camphor. There was a much slower onset of paralysis. One was almost completely paralyzed in three hours, the second slightly affected. On removal from water breathing recommenced, but the animals subsequently died. There was no evidence of reaction to light changes at any period.

Discussion of Results.

It is evident that the response of animals under a certain type of narcosis to illumination changes is widespread throughout marine species; this is probably true for land animals also. Our observations have been limited to species in which definite responses could easily be detected. More detailed study would probably reveal minuter changes, which, if employed, would show that the phenomenon is even more general. It is probable also that in certain cases

a greater range of dilutions of the chemical compounds employed would yield more definite results; *e.g.*, our results for certain species of shrimps are inconclusive, and we have been unable to demonstrate "darkness" responses with narcotized sea-urchins, though these have been reported for the normal animal.

In addition to the normal "shadow" responses quoted in the introduction may be mentioned here the well-known reaction of frogs to shadow (immediate diving) and that of cryptobranchiate nudibranchs (retraction of the gill-plumes within the branchial collar). Similarly *Euglena viridis* sinks.

The response itself is obviously adaptive (*i.e.*, of survival value, and so likely to have been accentuated by selection), since under natural conditions a shadow suddenly falling on an animal implies the interposition of a solid object between it and the light—*i.e.*, a probable enemy. It is extremely well developed in the pelagic fishes, as we found to our cost when trying to catch them. This normal response becomes less marked in all animals when examined in the laboratory under experimental conditions.

The peculiarity of the response in the narcotized animal seems to consist in its accentuation, actually, or relatively through the abolition of volitional movements and other reflexes, so that its manifestation can be controlled experimentally.

The reaction is always definitely a reflex, though the animal may be by no means in a condition of complete narcosis. While cessation of a very intense illumination undoubtedly produces greater effects than cessation of a lesser degree of it, the sudden change is the controlling factor, and is quite effective when made from a brilliant to a dull but distinct light. In almost all cases it is necessary to allow the animal to rest in dull light for a minute or two between tests, as after repeated subjection to strong illumination the response ceases. This is obviously connected with fatigue due to photochemical changes of the retinal pigment present alike in vertebrates and arthropods. While undoubtedly the retina is the receptor of the stimulus in animals possessing it, the normal reactions of sea-urchins suggest that their color spots can function in a similar way.

Increase of illumination either produces no effect or one much smaller than a similar decrease. There were one or two excep-

tions to this general rule. Thus *Calianassa,* a burrowing shrimp, gave positive reactions with "light" and none with "darkness." It is interesting to note in passing that certain animals normally living or moving in darkness appear to give such a reversed reaction. The well-known responses of the earthworm and of *Mya arenaria* may be quoted. But the subject can not be pressed further without more experimental evidence.

In a large number of cases the animal also responds to mechanical stimulation such as tapping the table on which the containing vessel rests at a period at which the retinal reflex becomes evident. The type of response is somewhat similar to that evoked by illumination changes and is often not yielded by the conscious animal subjected to the same slight stimulus. It nearly always ceases to produce a response much earlier than the "darkness" stimulus.

The results that we have recorded above have some interest from the pharmacological viewpoint. The action of the drugs employed is by no means similar in higher species (cf. Cushny[7]).

Camphor, in the frog, depresses the brain, and later the spinal cord, the action being a descending paralysis. There is no excitement, the reflexes gradually disappear, and the animal lies completely paralyzed. In mammals convulsions, due to stimulation of the higher areas of the nervous axis, are usually produced, and gradually pass into depression, stupor, collapse, and death from cessation of respiration, the cerebral cortex, medulla, and cord being in turn paralyzed. The terminations of the motor nerves are paralyzed in the frog by large doses (which may account for our failure to elicit the retinal reflex with camphor), but not in mammals. Menthol produces a similar series of phenomena, but there is less tendency to convulsions.

Phenol, in the frog, first causes depression and loss of spontaneous movements, later augmented reflex excitability, and finally tonic convulsions (due to increased irritability of the spinal cord). Complete paralysis of the central nervous system supervenes. Similar symptoms are produced in mammals, but there is often no preliminary stage of depression. The peripheral nerves and muscles are not affected in mammals and scarcely, if at all, in the frog. Benzene is stated to be much less toxic than phenol, though when

inhaled in large quantities it may give rise to similar symptoms. In some animals it produces violent and prolonged convulsions.

The differences of behavior exhibited by frogs and mammals to these drugs are shown by our results for the specific reflex that we have studied to be even greater when the observations are extended to include invertebrates. Although undoubtedly, as Cameron and Sedziak found for frogs, the degree of reaction differs markedly in different animals of the same species, and though our results were in some cases affected by the length of time that the animals were kept in the laboratory before they were tested, yet such marked variations are shown that a distinct idiosyncrasy of different species to the action of certain drugs is indicated. Thus camphor was little or non-toxic and did not reveal the reflex in cottoids and isopods; to the frog it was toxic, but the reflex could not be demonstrated; while with crabs, blennids, and pipe-fish marked responses to illumination changes were obtained with a varying degree of toxicity. Camphor was non-toxic for one species of shrimp, toxic but ineffective for a second, toxic and effective for a third; it gave marked reactions with the prawn employed by Fröhlich and Kreidl.

Quantitative comparison of the different drugs is difficult, not only on account of the different concentrations employed, but chiefly because of our ignorance of the rate of absorption of these drugs by different species and different sized specimens. The results for phenol and benzene can perhaps be contrasted most satisfactorily; it would seem that benzene is slightly less toxic, but it was of all the drugs employed the one most favorable for the production of the retinal reflex. It is to be noted that in at least one species (the isopods examined) for which camphor was non-toxic benzene was distinctly toxic.

It would seem that in most of the species studied benzene produces on the central nervous system an action distinctly comparable to the typical paralyzing action of phenol and camphor, and relatively much greater than that it produces in mammals.

Dr. Mary Rathbun, of the U. S. National Museum, kindly identified the species of shrimps employed, and Dr. B. W. Evermann, of the California Academy of Sciences, the blennids.

We desire to express our thanks to the Biological Board of Canada for the facilities placed at our disposal for the carrying out of this work.

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AN ANALYSIS OF THE SPAWNING HABITS AND SPAWNING STIMULI OF CHÆTOPLEURA APICULATA (SAY).

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The phenomenon of periodicity in the spawning activities of animals has attracted wide attention since its most striking example was made known in a series of papers by Collin, Kraemer, Friedlander, Izuka, and Mayer ('97–'08). These papers give the results of careful studies of the spawning habits of the Palolo Worm and show that both the Atlantic and Pacific species shed their gametes during particular phases of the moon and tides. The time of their swarming is so definite that it may be predicted as certainly as an eclipse.

Since these papers were published a distinct periodicity in the time of spawning has been revealed for a number of animals, related not merely to season, but to the time of the month. Many animals have long been known to spawn regularly at a certain time of the day or night. This paper does not deal with the problem of such diurnal periodicity, but only with lunar periodicity. Most of the animals which are known to exhibit a periodicity of the latter category are annelids, and the degree of perfection of their periodicity varies with that which is just discernible, as in *Nereis dumerilii*, to that of the Atlantic palolo, *Eunice fucata*, in which the swarming is restricted to a certain phase of the moon once a year. This curious phenomenon is not restricted to animals, but is shown quite as perfectly by the brown alga, *Dictyota dichotoma*, as described by Williams ('05), Hoyt ('07), and Lewis ('10). Some other species of the Dictyotaceæ also show a similar periodicity in the time of freeing their gametes.

PERIODICITY IN CHÆTOPLEURA.

When an attempt was made, in the summer of 1919, to obtain the eggs of *Chætopleura apiculata* for embryological study, it was

discovered that they could not be had at all times. They were obtained plentifully from July 12 to 20 and then spawning suddenly ceased. The writer naturally supposed that the end of the spawning season had been reached and that no more eggs could be expected that summer. However, experiments were continued at intervals of a few days, and at the approach of the next full moon eggs were again obtained. This time the period of spawning extended from August 8 to 11, inclusive. No eggs were obtained after the eleventh of August, although numerous attempts to induce further spawning were made. By referring to the table which follows, it will be seen that spawning began at the approach of full moon in July and continued thereafter for approximately one week (or until the third quarter), then ceased for a time to begin again in like manner at the time of the next full moon, thus indicating a distinct lunar periodicity.

TABLE.

Phase of moon.	Dates on which spawning occurred, summer of 1919.
Full moon.....	July 12.
	July 13.
	July 14.
	July 16.
Third quarter.....	July 20. Spawning active, apparently at maximum, many eggs laid by large proportion of females. Spawning began at 8.00 P.M. and continued until 2.00 A.M.
	August 8. Spawning occurred about 9.00 P.M. No eggs had been obtained during the preceding two weeks which included the new moon and first quarter.
Full moon.....	August 10.
	August 11.
	No eggs obtained after August 11, although several trials were made.

During the summers of 1920 and 1921 experiments were continued for the purpose of determining the limits of the spawning season and the relation of spawning activity to the cycles of the moon. As a result of these experiments the time limits of the spawning periods were found to be much less restricted than the data collected in 1919 indicated. In general, the data obtained during the last two years show that eggs may be had in small quantities at almost any time. By far the greatest spawning activity, however, comes at the approach of full moon and continues for ten

days or two weeks. This more active spawning period includes two or three days preceding full moon and continues until after the third quarter, or with slight abatement until two or three days after new moon. In 1921 the diminution of egg-laying came promptly at new moon. The periods of excessive spawning activity are followed by periods of low activity during which spawning is practically suppressed. These periods of low spawning activity extend roughly from new moon until the approach of full moon.

More specifically stated, there were ten-day periods in each of the months of June, July, and August, during which spawning activity was remarkable for the abundance of eggs extruded, and there were other periods between each of these high points when spawning activity was diminished.

The low spawning periods were somewhat more distinct in 1921 than in 1920. In both years approximately sixty per cent. of the eggs obtained were spawned between full moon and the third quarter, and perhaps seventy-five per cent. during the periods beginning three days before full moon and extending to the next new moon.

The data obtained during the three years agree except that the spawning season of the last two years extended over longer periods, and that the lunar periodicity was less marked than in 1919, when spawning was confined to a few days in each month.

A further comparison of the results obtained during the three years is interesting in that the total number of eggs secured during 1920 was almost double that of 1921 and very far in excess of that of 1919. There is no apparent reason for this variation in egg production from year to year, but similar observations on other species of animals are common.

It may not be amiss in this connection to point out that Lillie and Just ('13) have found spawning activities in *Nereis limbata* which are quite similar to those of *Chætopleura*, but the periodicity of the two species may or may not be identical.

In what is to follow reasons are given for believing that the spawning periods of *Chætopleura*, under natural conditions, are more restricted than the results of the experiments carried out under laboratory conditions indicate.

Spawning under Laboratory Conditions.

It may be that the spawning habit and presumably other behavior of animals under laboratory conditions is not a reliable criterion for normal behavior in nature. This comes out most clearly when all of the factors which influence spawning under artificial conditions are considered.

It may almost be stated as a general rule that sexually mature marine animals are likely to shed their ripe eggs when placed under abnormal conditions. It would seem, therefore, to be a futile, or at least an unnecessary procedure, to attempt to duplicate normal conditions in the laboratory as a means of inducing spawning. For instance, in order to secure the eggs of the small lamellibranch, *Cumingia,* it is only necessary to take them from the sand in which they live and expose them to the air for an hour or two, whereupon, when placed in a pan of quiet sea-water, they begin to spawn abundantly within half an hour. Eggs may be obtained in this manner at almost any time during the summer, and it is not uncommon for every female which is brought into the laboratory to spawn. It is not probable that many of these females would have spawned at these particular times if they had been left undisturbed in their normal situations.

Consider the case of the annelid, *Hydroides.* In order to cause them to spawn it is sufficient to break away their calcareous tubes and place them in sea water. Under these conditions they shed their sexual products within a few seconds. Dr. E. E. Just states that it is only necessary to pinch their tentacles or snip them off with scissors to induce spawning. They need not be removed from their tubes. It would appear, therefore, that the spawning of mature sexual products, in these cases, is influenced more by shock than by normal conditions. In fact, the more unnatural the conditions are made the better. However, after the shock has been applied, they require to be placed in a quiet situation, and presumably the more nearly it approaches normal conditions the better.

If these data have general significance, we may suppose that experiments which are performed in laboratories upon the behavior of animals, especially with reference to spawning, should be

checked up as far as possible by observations of their habits under natural surroundings where the possibility of shock is eliminated.

The writer is presuming, therefore, that the spawning periods of *Chætopleura* are restricted to ten days in each of the summer months, extending approximately from full moon to the third quarter. This, however, is not set forth as a fact. If laboratory data are wholly trustworthy, the spawning periods extend from full moon to new moon with the qualifications noted in the text.

Conditions Affecting Spawning.

As a matter of convenience in the study of the spawning periods of *Chætopleura*, it was found most advantageous to remove the animals from the shells to which they normally cling and to keep them in shallow crystallization dishes.

It was soon discovered that they spawn only at night, usually beginning about 7.30 or 8 P.M. and continuing until 10.30 P.M. It is not uncommon for them to spawn at intervals throughout the night.

During the day the animals were kept aërated by means of running water, but toward evening they were washed free from sediment and placed in dishes on laboratory tables. They rarely spawned in running water, but the change to quiet water furnishes an effective stimulus to spawning. Under these conditions one or more males usually respond within fifteen minutes, females somewhat later. If allowed to remain in running water, neither males nor females respond, so far as has been observed. It is therefore evident that these animals are affected by currents of water, and one naturally suspects that they spawn under natural conditions, either at low or high tide when the water is slack. No convincing evidence that they spawn at the time of low or high tide was obtained from laboratory experiments.

Although these animals rarely spawn in water that is strongly agitated, they spawn readily in dishes which constantly receive a fresh supply of sea-water, provided the inflowing stream does not perceptibly disturb or agitate the water in the dish. This shows that spawning is not induced by the accumulation of carbon dioxide. The same conclusion may be drawn from the fact that spawning

frequently begins within a few minutes after the animals have been placed in quiet water, and that it is practically over before the surrounding water is greatly affected by respiration. During the summer of 1920, at the height of the breeding season, a few females spawned in strongly agitated water. This occurred only once (July 8).

The number of eggs obtained from one female varies from about three hundred to three thousand, the average number being about sixteen hundred. As a rule only a few eggs are obtained during the first night after the animals have been collected. The greater number of females spawn the second night. A few spawn also during the third and fourth nights, but usually not to any considerable extent.

Spawning Stimuli.

Soon after being placed in quiet water the males begin to emit sperms in a double stream which soon become dispersed as clouds throughout the containing dish. The females respond more slowly after from one to three hours. One is therefore likely to suppose that something is expelled with the sperm which serves to stimulate the females to extrude their eggs.

This phenomenon was noted by M. M. Metcalf ('92) while studying two species of *Chiton* in aquaria. It was also described by Harold Heath ('05). F. R. Lillie and E. E. Just ('13) showed, without question, that in the case of *Nereis limbata* a substance is expelled with the sperm which is an effective stimulus to the female to shed her eggs promptly. In similar manner they found that the males will not shed their sperm except in the presence of a female which contains mature eggs or when placed in a dish of sea-water in which a mature female had been allowed to remain for an hour or more.

In order to test the assumption that this is also the case in *Chætopleura,* twenty mature individuals were washed thoroughly with fresh water and isolated in finger bowls of sea-water. During the night three of these females spawned and six males shed sperms in quantity, thus showing that neither males nor females derive any stimulus essential for spawning from the opposite sex. It is evident that the females were not induced to spawn by the

presence of the spermatic fluid from the fact that none of the eggs initiated development. After some hours had elapsed these eggs were artificially fertilized and a considerable percentage of them went through cleavage stages, showing that they were normal, mature eggs, and that they had not previously been subjected to spermatozoa.

This experiment, first performed in 1920, was verified during the past summer.

Both sexes are apparently affected by the change from running to quiet water (tide changes), presumably also by the change in pressure between high and low tides (mechanical shock), and most certainly by moonlight.

It is evident that these factors have not been analyzed satisfactorily, but Mayer ('07 and '08), by actual controlled experiments, has contributed at least a first step toward a solution. The writer is of the impression that Scott's ('09) explanation, in the case of *Amphitrite*, that the periodic maturing of the sexual products is due to the warming effect of the sun at periods of especially low tides, can not be generally applied and is not applicable to *Chætopleura*.

The non-committal statement made by Lillie and Just ('13), that the maturing of the sexual elements of these animals is dependent upon phases of the moon, involving, through lunar tidal variations, rhythmical alterations of conditions of nutrition, is less open to criticism and is as exact as the known facts warrant at the present time.

Many interesting suggestions concerning the cause of periodicity in spawning have been made by various authors, but most of them are undoubtedly wide of the mark.

Friedlander ('98–'01), after reviewing various possibilities, finally admits that he is wholly in the dark and arrives at the conclusion that no theories yet proposed are adequate to explain periodicity in spawning.

Mayer ('07 and '08) states, upon the authority of experimental data, that moonlight is the effective cause, and that the tides are unnecessary but contributing causes.

Hemplemann ('11) believes that the moonlight, acting through a period of several days, is the stimulus to the maturing of the

sexual products, and that it may also be the immediate cause of swarming.

A theory proposed by Brunelli and Schroener,[1] that the swarming has its origin in original accidental shocks to the nervous system of these worms, and that their swarming activities finally became spontaneous or hereditary in a form that was favorable to the propagation of the species, is probably true, but it explains nothing. That the habit of spawning periodically has become hereditary is shown by the fact that the animals under discussion will spawn at the proper time even when the normal stimuli are altered or absent. See Mayer ('07).

Most of these theories are based upon abstract reasoning and not upon experimental data. It is apparent that there is something yet to be done before we can understand this curious phenomenon and its relation to the moon. That this habit has become more or less hereditary is shown by several authors. However, it must have originated in relation to external stimuli which are felt periodically. The cause of this type of periodicity must be fundamentally the same in all cases, although it is exhibited in a variety of ways, and at different times relative to the phases of the moon.

Experiments on Chætopleura.

Summer, 1920.

I.

June 20–23:

20 Chitons collected June 20.

1st night, no eggs.

2d night, no eggs.

3d night, no eggs.

* First Quarter, June 23.

II.

June 24–27:

30 Chitons collected June 24.

1st night, no eggs, some spermatozoa.

2d night, no eggs.

3d night, no eggs.

* It is due to the last two authors mentioned to state that the writer has read only quotations from their papers.

III.

June 27–30:

15 Chitons collected June 27.

1st night, four females laid eggs; spermatozoa abundant.

2d night, two or three females spawned lightly.

3d night, two or three females spawned lightly.

IV.

June 28–30:

100 Chitons collected June 28.

1st night, no eggs.

2d night, great quantities of eggs from 12 to 15 females; spermatozoa abundant; eggs laid 9–12 P.M.

3d night, two females spawned in quantity; a few eggs from several others.

4th night, two specimens spawned.

5th night, a few still spawned.

Note.—Nearly all spawned second night. Spawning is nearly always on 1st or 2d night after collecting. Spermatozoa appear first in every case observed.

V.

*Full moon, July 1.

July 2–3:

20 Chitons collected July 2.

1st night, two spawned, one of these about 10 P.M.

2d night, two spawned; spermatozoa shed in abundance as soon as animals were placed in quiet water.

VI.

July 3–4:

158 Chitons collected July 3. Placed 25 per dish.

1st night, two spawned.

2d night, about 20 females spawned, and about 30 or more males shed spermatozoa. (This group continued to spawn freely for five nights.)

VII.

July 6–8:

100 Chitons collected July 6.

1st night, several specimens spawned about 10 P.M., and a few more after 1 A.M.

2d day, several females spawned between 9 and 10 A.M., July 8, and one or two continued at intervals during the afternoon; spermatozoa also abundant. Beginning about 5 P.M., many females (35–40) spawned in great abundance, laying 2,000–2,500 eggs each.

Note.—July 8 seemed to be the height of the spawning season. Spawning occurred day and night, practically all night and more or less during the entire day. Spawning during the day not seen before.

* Last Quarter, July 8.

Note.—Experiments so far show that females spawn at the approach of full moon, a few days before. Spermatozoa are shed for longer periods and more readily than the eggs. Spermatozoa appear in clouds very soon after animals are placed in quite water, 5–10 minutes, and sometimes continue nearly all night. Females sometimes spawn the first night after collecting, but more abundantly the second night.

VIII.

July 11–14:

100 specimens collected July 11.

1st night, several specimens spawned.

2d night, no eggs.

3d night, several specimens spawned.

4th night, several specimens spawned.

Note.—This group was not well cared for, because attention was necessarily diverted to other matters. There was considerable if not abundant spawning, although not comparable to that of July 8.

* New moon, July 15.

IX.

July 17–18:

100 specimens collected July 17.

1st night, two specimens spawned.

2d night, several specimens spawned in quantity, 7–10 P.M.

* First Quarter, July 22.

X.

July 22–25:

100 specimens collected July 22.

1st night, three spawned about one half usual amount of eggs.

2d night, about six specimens spawned in quantity, one half to two thirds maximum.

3d night, two specimens spawned lightly and a few eggs from others.

4th night, none spawned.

XI.

July 28–30:

75 specimens collected July 28.

1st night, one spawned; a few eggs from a second.

2d night, about ten specimens spawned in great abundance, some about three thousand eggs.

* Full moon, July 30.

XII.

August 4–6:

75 specimens collected August 4.

1st night, very little spawning.

2d night, about eight spawned in abundance.

XIII.

* Last Quarter, August 7.

August 7, experiment worthless. Chitons suffocated by standing without water.

XIV.

August 10–11:

120 specimens collected August 10. Good lot.

1st night, no eggs.

2d night, three specimens spawned 8–10 P.M.; almost none after 10 P.M.

* New moon, August 13.

XV.

August 20–22:

112 specimens collected August 20.

1st night, no eggs and no spermatozoa.

2d night, one female spawned lightly (300 eggs), also twenty eggs from another, and nine from a third.

3d night, one female spawned average number of eggs (1,500), two others about 300 each.

XVI.

August 24–26:

94 specimens collected August 24.

1st night, no eggs, no spermatozoa.

2d night, two males emitted clouds of spermatozoa; one female spawned good bunch of eggs, a second thirty-two eggs.

3d night, three females spawned lightly (100 to 300 eggs each).

Remark.—Very light spawning since new moon.

* Full moon, August 29.

XVII.

August 28–31:

30 Chitons collected August 28. Left in supply department in insufficient amount of water until 10 P.M. (Fault of collectors.)

1st night, experiment set at 10 P.M.; no eggs and no spermatozoa.

2d night, two females spawned lightly and one male emitted spermatozoa.

3d night, two females spawned maximum quantity of eggs, 2,500 each.

4th night, no eggs.

Note.—The last experiment is more or less untrustworthy, because the chitons were partially suffocated by improper treatment after collecting. The group, however, shows a distinct revival of spawning in that thirty chitons spawned many times more eggs than the larger group of the preceding experiments.

Experiments, 1921.

Experiments on Chætopleura. Summer of 1921.

* Full moon, June 20.

I.

Experiment 1, June 24–26.

1st night, of twenty-five large chitons collected June 24, three and possibly four spawned in fair quantity, first night, perhaps 50–60 per cent. of maximum; none spawned before 10 P.M.

2d night, spawning began at 10.30 P.M.; probably over before midnight, as indicated by development of embryos next morning; five females out of twenty-five animals spawned one half to two thirds maximum; good quantity.

Remark.—Experience of last year has shown that most of the spawning is done the second night after collecting. Therefore, it is best to consider only 2d night in studying spawning habits, or else compare experiments as a whole.

* Last Quarter, June 28.

II.

Experiment 2, June 30–July 2.

30 specimens collected June 30.

1st night, no females spawned; a few males shed spermatozoa (2 or 3).

2d night, three females spawned about one half maximum.

3d night, two females spawned in considerable quantity, a third one lightly.

Remark.—Impression of good spawning activity during the last week in June. Plenty of eggs for embryological study. Isolated specimens also spawned readily and in quantity.

III.

Experiment 3, July 2–5.

60 specimens collected Saturday, July 2; 30 placed in running water and 30 placed, as usual, on laboratory tables.

1st night, none spawned in either case; one male was shedding spermatozoa at 7.30 A.M. Sunday morning.

2d night, of those placed in running water one spawned one fourth maximum; of those placed on laboratory table two spawned lightly; several males shed spermatozoa in quiet water (abundant from 8 to 11 P.M.); very little in running water.

3d night, those placed on laboratory table spawned about 25 eggs; those placed in running water spawned 12 eggs; one specimen spawned in each case; males shed spermatozoa in considerable quantity in quiet water.

Remark.—Very light spawning from this group.

* New moon, July 5.

IV.

Experiment 4, July 5–7.

40 Chitons collected July 5. Placed in running water promptly, but not set for experiment night of July 5.

2d night, set in two lots, one in running water and one on laboratory table in quiet water; twenty good specimens each; of those on laboratory table one spawned 100 eggs more or less; of those in running water two spawned not to exceed 100 eggs each, between 9–11 P.M.

3d night, set as above; none spawned during the night, males nor females.

Remark.—Almost no spawning from this group.

V.

Experiment 5, July 7–9.

80 Chitons collected in afternoon, July 7. Divided into two lots, 40 each. Medium size to large specimens. Good lot. One group placed in running water and one in quiet water on laboratory table, as usual, about 7 P.M.

1st night, none spawned in either dish, males nor females.

2d night, eggs laid by two females in quiet water, about 100 eggs each or one tenth maximum; spawned at 11 P.M.; none laid by those in running water; one male shed great cloud of spermatozoa in quiet water.

3d night, no eggs and no spermatozoa obtained in either dish.

Remark.—Spawning at this time and for several days past practically suppressed.

* First Quarter, July 11.

VI.

Experiment 6, July 12–14.

60 Chitons collected in afternoon, Tuesday, July 12, and kept in running water at supply department night July 12.

2d night, July 13, experiment set in two dishes as usual; of those in running water none spawned; of those in quiet water on laboratory table three males shed spermatozoa freely before 8 P.M., and others during the night; one female spawned lightly one fourth the maximum amount about 10 P.M.; about thirty-five additional eggs were spawned by one female during the night.

3d night, at 10.30 P.M. a few males shed spermatozoa abundantly; one female spawned and only 40–50 eggs in quiet water; one female in running water spawned about 30 eggs during the night.

Remark.—Spawning practically suppressed.

VII.

Experiment 7, July 14–16.

90 Chitons collected afternoon of Thursday, July 14. Placed about 6 P.M. in three dishes. One left in running water. Two placed in quiet water on laboratory table.

1st night, spermatozoa in small amount shed by three males before 10 P.M. in quiet water; no eggs in either dish up to 11 P.M.; about 40 eggs obtained from one female in quiet water during the night.

2d night, experiment set as usual 6 P.M.; those in quiet water began to shed spermatozoa about 7.30 P.M. (one male); between 7–10 P.M. three females spawned in quantity two thirds maximum in one dish; three others spawned in quantity in another dish (about 30 chitons per dish); no eggs obtained from dish in running water.

3d night, no eggs obtained; experiment not set until 10 P.M.

Remark.—Spawning distinctly revived. For ten days preceding no eggs obtainable for class work. Was unable to get eggs for embryology class during first half of July. Spawning revived three days before full moon.

VIII.

Experiment 8, July 16–19.

1st night, about 50 chitons placed in crystallizing dish on laboratory table at 10 P.M. (too late). (Collected during afternoon of July 16.) No eggs first night.

2d night, five specimens spawned rather lightly one fourth maximum; one mass of considerable size, three fourths maximum or more; eggs in addition scattered all over the bottom of the dish.

3d night, two or three females spawned lightly before 10 P.M.; very few eggs; one fair-sized group.

4th night, no eggs.

Remark.—Spawning by this group was not particularly heavy. More eggs were spawned by previous group July 15. Not a great difference and not inconsiderable.

* Full moon, July 19.

IX.

Experiment 9, July 20–22.

40 specimens collected July 20. Placed on laboratory table at 6 P.M. Three females spawned lightly before 10 P.M. About 50 eggs each. None after 10 P.M.

2d night, placed on laboratory table at 7 P.M.; three or four spawned one third to one half maximum or more before 10.30 P.M., mostly before 9 P.M.; spermatozoa abundant; none after 11 P.M.

3d night, four females spawned about twenty eggs each; practically none at all.

Remark.—Only fair spawning activity. Plenty of eggs for class, however, and approximately equal to the two preceding experiments, 7 and 8. Spermatozoa were abundant.

X.

Experiment 10, July 25–26.

70 specimens collected Monday, July 25. Mostly half or two thirds grown, but sexually mature.

1st night, five spawned, one half to two thirds maximum.

2d night, six or seven spawned; four of them one half to two thirds maximum; impression of fair or good spawning activity; good quantity as spawning goes this year; perhaps as heavy as the best this season; comparable to that of the last week in June, but probably not quite equal to it.

* Last Quarter, July 27.

XI.

Experiment 11, July 27–29.

75 Chitons collected Wednesday, July 27. Placed in two dishes upon laboratory table. None spawned 1st night, except a very few eggs from two or three individuals. Negligible.

2d night, six specimens spawned about one third to one half maximum; all spawned before 10 P.M.; mostly between 8–10 P.M. (There were only 45 specimens used this 2d night. Several had been lost.)

3d night, no eggs.

Remark.—Good spawning activity.

XII.

Experiment 12, August 1–3.

100 Chitons collected Monday, August 1.

1st night, placed in three dishes on laboratory table; three females spawned very lightly before 10 P.M. about one fifth maximum or less; none after 10 P.M.

2d night, two dishes placed on table; one left in running water; about 75 specimens in all, some having been lost; four females spawned about 25 eggs each by 10 P.M.

3d night, no eggs.

Remark.—Spawning greatly reduced and all but suppressed. Spawning this year much lighter than last year.

XIII.

Experiment 13, August 5–6.

40 Chitons collected Friday, August 5.

1st night, placed in two dishes at 7 P.M.; no eggs or only one half dozen eggs from one female; one male shed spermatozoa before 10 P.M.

2d night, four females spawned about two thirds maximum and a fifth one half maximum; impression of fair spawning activity; good for this year; all done before 11 P.M.

3d night, about 25 eggs from each of three females; practically none; done before 11 P.M.

* New moon, August 3.

XIV.

Experiment 14, August 9–10.

60 Chitons collected Tuesday, August 9, but not brought to laboratory until next day.

2d night, two females spawned about one fourth maximum.

XV.

Experiment 15, August 10–12.

25 Chitons collected August 10—Wednesday.

1st night, none spawned.

2d night, two spawned one fourth maximum; *very light.*
3d night, one female spawned about one sixth maximum.

Remark.—Spawning very light. Almost none.

*First Quarter, August 10.

XVI.

Experiment 16, August 12–14.

45 Chitons collected Friday, August 12. Placed in two dishes.
1st night, none spawned.
2d night, three females spawned (two one fifth maximum; one one half maximum; two in one dish and one in the other); a fourth specimen spawned 50–100 eggs after 10 P.M.
3d night, no eggs.

Remark.—Impression of light spawning activity. Spawning all but suppressed during past week. Quantity of eggs not sufficient for embryological studies.

XVII.

Experiment 17, August 15–17.

100 Chitons collected Monday afternoon. Arrived 5 P.M. Put in dishes 7.30 P.M. Four dishes.
1st night, no eggs.
2d night, abundance of spermatozoa shed; water clouded; also quantities of eggs from three to five females in each dish one third to two thirds maximum.
3d night, August 17, two females spawned one fourth maximum; spermatozoa very abundant, but very few eggs.

Remark.—Very distinct revival of spawning activity; most abundant this year.

*Full moon, August 18.

XVIII.

Experiment 18, August 18–20.

50 large Chitons collected August 18.
1st night, animals remained in aquarium in laboratory (not discovered).

2d night, set in two dishes on laboratory tables; one female of medium size spawned maximum quantity (approximately 3,000 eggs); two others spawned one sixth to one fourth maximum; a fourth laid a few eggs, all before 10 P.M.; an enormous quantity of spermatozoa shed; white cloudy water.

3d night, August 20, no eggs; small amount of spermatozoa.

Remark.—Not many females spawned, but the greatest mass of eggs obtained during this year came from one female of this group. Spermatozoa were particularly abundant. An abundance of spermatozoa has been noted at each full moon, water becoming so turbid that one could not see eggs on the bottom of the shallow dish.

XIX.

Experiment 19, August 22–23.

55 Chitons collected Monday, Aug. 22.

1st night, no eggs.

2d night, only about 50 eggs from a single female; no visible spermatozoa.

Remark.—Practically no spawning.

XX.

Experiment 20, August 24–27.

60 specimens collected Wednesday, August 24.

1st night, no eggs.

2d night, one female spawned two thirds maximum between 8–10 P.M.; one male shed spermatozoa; two other females shed eggs before 11.30 P.M., about 300 and 100, respectively; a fourth female spawned during the night after 11.30 P.M. one half to two thirds maximum.

3d night, two spawned one third maximum before 10 P.M.

Remark.—Fair spawning activity.

* Last Quarter, August 26.

XXI.

Experiment 21, August 26–28.

Chitons collected from both Nobska Light and Vineyard Haven, August 26. About 100 specimens.

1st night, no eggs.

2d night, no eggs up to 10 P.M.; one female spawned 20 eggs after 10 P.M.

3d night, one female spawned 100 eggs.

Remark.—Practically no spawning. Many specimens used. Season rather late. Spawning sporadic.

XXII.

Experiment 22, August 29–30.

60 Chitons collected August 29.

1st night, no eggs.

2d night, six females spawned very lightly one fifth to one tenth maximum; only a few eggs each; several males shed spermatozoa.

Remark.—Represents a distinct increase over preceding nights. None after 10.30 P.M. Spawning per individual pretty light, however.

XXIII.

Experiment 23, August 31–September 1.

120 Chitons collected August 31. Placed in three dishes.

1st night, no eggs.

2d night, nine females spawned; three one half maximum, others one fourth to one fifth maximum; very considerable spawning and much spermatozoa in one dish; two dishes with little spermatozoa.

3d night, no eggs.

Remark.—Good spawning activity for time of season.

* New moon, September 1.

XXIV.

Experiment 24, September 6–7.

85 Chitons collected Tuesday, September 6. Placed in three dishes.

1st night, no eggs.

2d night, two females spawned in one dish two thirds maximum; one spawned in another dish one fourth maximum, 8–10.30 P.M.; third dish, no eggs.

Remark.—Experiments for the season closed September 7. For some days there had been indications that the spawning season was nearing its end. Impression of fair spawning activity, although from only a few individuals. The experiments show that heavy spawning begins within a few days of full moon and continues to third quarter, or a few days after third quarter. The lightest spawning occurs between new moon and full moon, especially pronounced around first quarter.

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NOTES ON THE LOCOMOTION OF THE NUDIBRANCHIATE MOLLUSK DENDRONOTUS GIGANTEUS O'DONOGHUE.*

H. P. KJERSCHOW AGERSBORG.

Many works have already appeared on molluscan locomotion. Thus Simroth ('79) called attention to the relation between the pedal nerve, the musculature of the foot, and the "locomotorischen Wellen" in *Limax*. Fleischmann ('85) observes: "Kontraktionswellen über die ganze Oberfläche des Fusses in *Anodonta*, hinten beginnend und nach vorn strichend." Jordan ('01), and again in 1905, records a similar feature for *Aplysia limacina*. Bohn ('02) finds that progressive movements of *Helix pomatia* L. are accomplished by undulations in the form of waves of the pedal musculature which rests against a solid or support, and that these rhythmic pedal waves are partly independent of mechanical excitations. Künkel ('03) finds that the "Wellenspiel" increases when he touches a crawling *Limax tenellus* (or *L. agrestris, L. arborum*). Carlson ('05) substantiates the findings of Jordan ('01) by describing for *Helix dupetithonarsi* a succession of large waves which pass from the head to the tail, an extraordinary mode of progression employed when the animal is in a hurry. Biedermann ('05) refers to "Querbänder" that follow one another in parallel lines on the under side of the foot of *Helix pomatia*, when this species crawls on a glass plate. Von Uexküll ('09) confirms the findings of Jordan for *Aplysia*, viz., that waves are advancing, when the foot is lifted from the ground, from the anterior to the posterior, the waves advancing in the opposite direction in *Limax* and *Helix*.

A more complete analysis of pedal locomotion among mollusks and other invertebrates has been accomplished by the brilliant works of Parker ('11, '14, '17, '17*a*, '21) and his pupil, Olmsted ('17). Parker ('11) recognizes two main sets of locomotion means for ordinary gastropods: (1) arhythmic (*Ilyanssa obsoleta*

* Contribution from the Zoölogical Laboratory of the University of Illinois, No. 200.

Say) locomotion is accomplished without pedal waves; (2) with pedal waves, or rhythmic locomotion (*Dolabrifera virens* Verrill, *Tectarius nodulosus* Gmel., *Nerita tessellata* Gmel., *Chiton tuberculatus* Linnæus, etc.). "In rhythmic locomotion the waves may run from the posterior to the anterior, that is, *direct;* or the reverse, that is, *retrograde.* The foot may exhibit one, *monotaxic;* two, *ditaxic;* or four, *tetrataxic,* series of waves. In the ditaxic foot the waves may be alternate or opposite. . . . Locomotion is the cumulative result of local forward motion on the part of one section of the foot after another till the whole foot has been moved." The essential act of pedal locomotion of all creeping gastropods may be exhibited by the retrograde wave movement on the foot of *Chiton tuberculatus,* in which, according to Parker ('14), the locomotor waves run from anterior to posterior. In this type, "The waves extend the whole width of the foot and are from five to six mm. in antero-posterior extent. They represent an area of the foot temporarily lifted from the substrate. . . . Excepting in the region of the wave, the foot is firmly attached to the substrate; hence at any moment from nine tenths to four fifths of the foot is fixed and the remainder free." Pedal locomotion of Actinians (*Metridium marginatum* Milne-Edw., *Sagartia luciæ* Verrill, *Condylactis passiflora* Duch. and Mich., and *Actinia bermudensis* Verrill) is interestingly accomplished by a wave-like movement which progresses over the pedal disc in the direction of locomotion (Parker, '17). "In the actinian locomotor wave each point on the pedal disc is successively raised from the substratum, moved forward, and put down." Writing on the locomotion of the sea-hare, *Aplysia californica* Cooper, this same author ('17*a*) says: "Pedal locomotion in *Aplysia* is due to monotaxic retrograde waves which lift the foot locally and temporarily from the substrate, making it thus to move forward with freedom, while the rest of the foot for the time being holds the snail in place by many small areas of local suction. The portion of the foot that moves forward is the elevated region," pp. 143 and 144. Locomotion in the holothurian *Stichopus panimensis* Clark is effected by direct monotaxic waves (Parker, '21). That is, "Creeping is accomplished in part by a muscular wave that originates at the posterior end of the animal and sweeps over it to the anterior." It is inter-

esting to note that locomotion in this echinoderm is in essentials of that type of gastropod locomotion which has been designated the direct monotaxic type.

Olmsted ('17) finds in the huge slug *Veronicella schivelyoe* Pilsb. there are always about eleven pedal waves which pass from the posterior to the anterior and extend the full width of the foot; this is direct monotaxic locomotion. In *Onchidium floridanum* only one or two waves are shown at a given time. In *Eulota simularis* Fer. the average number of waves are 9 à 10. *Helcinia convexa* Pfr., *Tethys dactylonula* Rang, and *Fissurella nodosa* Born show retrograde monotaxic locomotion. *Tectarius misricatus* L. exhibits retrograde alternate ditaxic locomotion. *Tritonidea tincta* and *Columbella mercatoria* L. are retrograde tetrataxic in their locomotion. *Cypræa exanthema* L. shows long and short lateral waves which move either to the right or left; complete diagonal waves which move to the right or left, and retrograde waves from the anterior edge to the center of the foot and extends to the posterior. Finally, *Marginella arena* Val., *Haminea antillarum* Orb., and *Bulla occidentalis* A. Ads. move by ciliary action alone. Retrograde pedal waves, according to Crozier ('19), are the means of locomotion in *Ischnochiton purpurascens* Ad., *Acanthochites spiculosus* Reeve, and *Tonicia* sp.; the first-named one also exhibits a "gallop" like that of *Helix*, which is independent of the pedal waves.

The remarkable nudibranch *Dendronotus giganteus* has recently been described (1921) from the Vancouver Island region by Dr. Chas. H. O'Donoghue. This author records four species of this genus: *D. arborescence* Müller, *D. dalli* Bergh, *D. giganteus* O'Donoghue, and *D. rufus* O'Donoghue, from this region. The largest of these species measured: 50 mm. long by 15 mm. high by 15 mm. broad; 31 mm. by 9 mm. by 6 mm.; 140 mm. by 43 mm. by 33 mm.; and 14 mm. by 4.5 mm. by 4 mm., respectively. According to this author, a preserved specimen of *D. giganteus* in the laboratory obtained by Professor C. M. Fraser, 1913, measured 210 mm. long by 84 mm. high by 55 mm. wide. Allowing for approximately the same amount of shrinkage, according to O'Donoghue, the specimen when alive must have reached the size of 260 mm. long by 100 mm. high and 65 mm. broad. This is indeed a

gigantic size for a nudibranch! It is matter of common knowledge, however, that the fauna of the North American Pacific coast attain not only a large size, but are in fact very abundant and diversified. (*Vide:* Kjerschow Agersborg, 1920.)

Large specimens of this genus also occur in the vicinity of Puget Sound Biological Station, Friday Harbor, Washington. Thus during the summer of 1913 a collecting party from the station found on the shore of Shaw Island (not very far from Canoe Island) a large specimen which must have measured about 200 mm. in length. To the writer's knowledge no one identified the specimen at that time. It was conveniently classed *D. arborescence.*

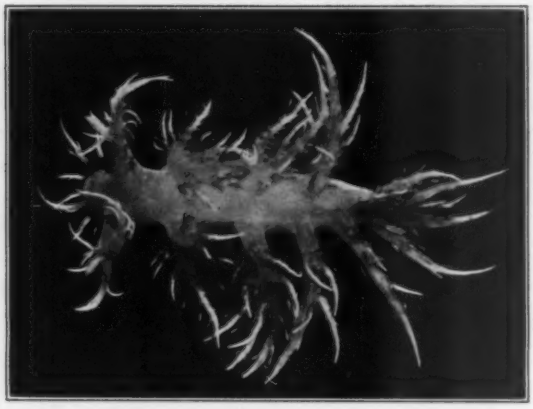

FIG. 1. Photograph of *Dendronotus giganteus* O'Donoghue, dorsal view.

During the summer of 1921 I again visited the Puget Sound Biological Station. On July 20 I found a very fine specimen of *Dendronotus* between the logs of the floating dock of the station (Figs. 1–4). Upon examination it was found to fit perfectly to the description of O'Donoghue for *D. giganteus.* It measured 140 mm. long by 60 mm. high by 40 mm. broad. The foot was 90 mm. long and 40 mm. wide at its widest part. This specimen was kept alive in the laboratory for three weeks. During this time it did not feed, as far as I know, on anything. I tried to feed it on crustaceans, echinoderm gonads, cœlenterate tentacles, green algæ,

etc., but it refused all these except once, in which case it opened its large fleshy lips in response to the touch of cœlenterate tentacles, took them within its mouth, only to be ejected a little later. When the animal was first found, and for several days afterwards, its stomach was filled with air, which aided it in floating. It was

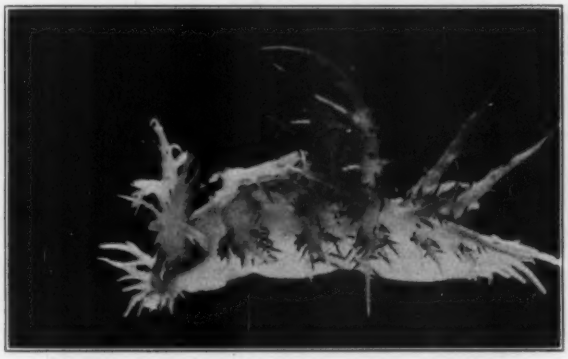

FIG. 2. Photograph of *Dendronotus giganteus* O'Donoghue, lateral view.

able, however, to submerge itself while in this condition. When the air bubbles contained in the stomach after a few days had disappeared, a little by little, the animal was able to float nevertheless.

In conjunction with the study of qualitative chemical and physical stimulations in *Hermissenda opalescence* Cooper, I also studied *D. giganteus* (*vide* Kjerschow Agersborg, 1922*a*; for a full description of the species: O'Donoghue, 1921), and noticed incidentally its remarkable mode of locomotion. During the first few days in the laboratory it was very active, and it was quite difficult to make proper observations on its response to stimuli; on the other hand, it offered the opportunity to study its mode of locomotion which is appended.

Dendronotus giganteus has two distinct modes of locomotion. The one, and the most commonly used, is that of swimming; the other is creeping. The swimming movements are effected by a regular twisting of the body in an undulatory manner, beginning at the anterior end and passing gradually to the posterior. These

movements are alternated from side to side. The lashing of the anterior part of the body from side to side is relatively powerful and a much more rapid progressive movement is effected over that attained by creeping. The undulatory movements employed in swimming start with the head being bent downward and sideways, forming a wave-like twist in the side of the body-wall like that in a blade of a propeller. This wave passes gradually toward the posterior end (Figs. 3–4), and disappears when the animal makes

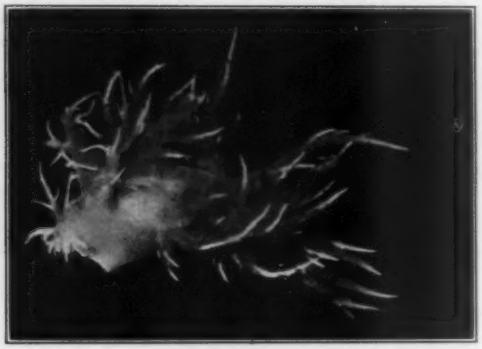

FIG. 3. Photograph of *Dendronotus giganteus* O'Donoghue in the act of swimming; the head has returned from its bend to the right, and the twist-like wave is shown in the side passing toward the posterior end.

the next stroke to the opposite side. When the animal makes a stroke to the right the posterior two thirds is bent so as to form an angle of 45° with the anterior one third. But the posterior part of the body also rotates about 45° from the vertical plane, so that the left side with the foot forms a large wave which sweeps posteriorly, while the anterior part of the body, in front of the wave, is kept vertically. When the animal makes a stroke to the left, the same phenomenon is repeated on the right side. An animal may cover a distance of 30 cm. in a few seconds, making side-strokes of about 45 a minute.

The creeping method of locomotion was seldom employed. If the animal was forced to the bottom of the dish, it would attach itself to the substratum and commence gliding along imperceptibly. Close observation brought to light, however, a succession of undu-

lations which passed from the posterior end of the foot to the anterior. The animal adhered to the substratum by sucking to the extent that it did not pass out of the dish when the water was poured out of it. In creeping, local undulations are set up in the

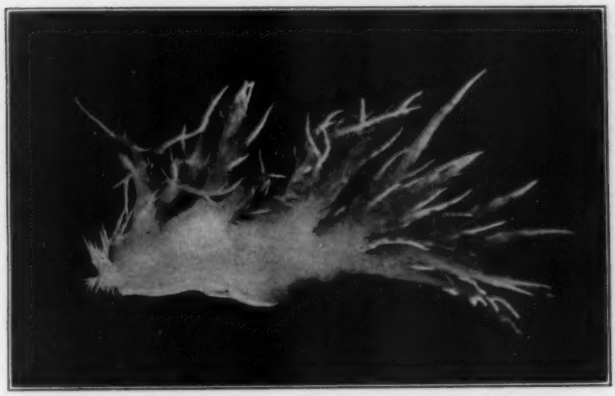

FIG. 4. Photograph of *Dendronotus giganteus* O'Donoghue showing the locomotor wave during swimming passing toward the posterior end. All the photographs are of living specimens, photographed through the water, by Dr. Myrtle E. Johnson, State Teachers College, San Diego, Calif. The photographs were taken at the Puget Sound Biological Station, July, 1921.

form of series of areas adhering to the substratum, and intervened by non-adhering parts which travel gradually toward the anterior end. The method of creeping may be classed as direct rhythmic locomotion, to use the terminology of previous writers on this subject, although it is not possible to classify this exactly at this time. The method of rhythmic locomotion is accompanied in this case, I believe, by ciliary action. The foot in *Dendronotus giganteus* is highly ciliated. No slime is produced during locomotion or at any other time. The foot is also uniformly ciliated in *Melibe leonina* Gould (Kjerschow Agersborg, 1921*a*, 1922). Ordinary locomotion in *Melibe* is effected by the ciliary action of the foot. The ciliated epithelium is innervated with nerve fibers from the pedal nerve-net (Kjerschow Agersborg, 1922).

Swimming as a means of locomotion is common among pelagic forms which are then frequently provided with secondary organs

for that purpose as in *Aplysis* Linnæus, in which the parapodia figure quite prominently. Forms closely related to the nudibranchs, such as the gymnosomatous *Pteropoda,* may be particularly exemplified in this connection. These parapodic forms are pelagic *per se* (*vide* Kjerschow Agersborg, 1922*c*). Although several nudibranchs are pelagic, this habit is secondary, notwithstanding.

Collingwood ('79) records swimming as a means of locomotion in *Scyllea pelagica,* and Garstang ('90) for *Lomanotus* Verany. Kjerschow Agersborg ('19, '21, '22, '22*b*) reports swimming as one mode of locomotion in *Melibe leonina.* But none of the forms as here mentioned are good swimmers although Garstang's statement seems to indicate that *Lomanotus* progresses through the water during swimming. I am unaware of any previous record as to the pelagic habit of *Dendronotus.* The assumption may be quite justified that *Dendronotus,* in spite of its highly developed foot, is pelagic in habit, not only because of the evidence connected with the place of discovery of the particular specimen upon which this discussion is based, but rather on the striking, highly developed method of locomotion, viz., swimming. *Melibe leonina,* although quite frequently pelagic in habit, is not such an able swimmer as *Dendronotus giganteus.* The last-named species may, therefore, be more pelagic in habit than it is commonly thought to be.

Simroth, Fleischmann, Jordan, Bohn, Künkel, Carlson, Biedermann, von Uexküll, and others have shown that during ordinary locomotion not only do the muscle fibers of the foot take part in the production of the pedal waves, but also certain muscle fibers of the body-wall. This is more readily understood when one realizes the relation of the muscle fibers of the body-wall to those in the foot, in terrestrial as well as in aquatic or marine snails. The swimming habit, of course, is not equally developed in all, which is partly due to the general shape of the body. During swimming the muscle fibers of the body-wall act preëminently; during creeping, those of the foot.

Summary.

1. *Dendronotus giganteus* O'Donoghue swims by bending the anterior end of the body sideways to an angle of about 45°, pro-

ducing each time a powerful stroke by the head, and forming in the right or left side of the body-wall, according to the bend of the head, a large twisted muscular wave which passes toward the posterior. By the sweep of these waves locomotion is effected. Of all known non-parapodic nudibranchs, *Dendronotus giganteus* is the most able swimmer.

2. Creeping is effected by direct rhythmic waves augmented by ciliary action of the foot.

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NON-CRISS-CROSS INHERITANCE IN DROSOPHILA MELANOGASTER.

LILIAN V. MORGAN.

A complete reversal of the ordinary criss-cross inheritance of recessive sex-linked characters occurs in a line of *Drosophila* recently obtained. In ordinary sex-linked inheritance the recessive sex-linked characters of the mother are transmitted to the sons, while the daughters show the dominating allelomorphic characters of the father. In the present case the daughters show a recessive sex-linked character of the mother and the sons show the dominating allelomorphic character of the father. The reversal is explicable on the assumption that the two X-chromosomes of the mother are united and behave at reduction as a single body. Sections show that the eggs of these females do have two united X-chromosomes; the cytological evidence verifies the genetic deduction.

A female fly (Fig. 1, *a*), which was a somatic mosaic, showing different sex-linked characters in the anterior and posterior parts, appeared in a pair culture of which the parents had the following constitution: one X of the mother carried the differentials for eosin, cut and forked; the other X carried the differentials for forked and bar. She was therefore forked, heterozygous bar, and gray. The X of the father carried the differential for yellow body color, and he was therefore yellow.

The head and thorax of the mosaic (Fig. 1) were gray and the eyes were slightly bar, while the abdomen was yellow; the fly was entirely female, having heterozygous bar eyes, no sex-combs, and normal female genitalia. She was not virgin, but nevertheless was isolated and mated to a black male, and produced 43 daughters and 59 sons. The daughters were, without exception, all yellow and the sons were all gray, and all the offspring had wild type eyes—*i.e.*, none were bar except two sons that were also forked and will be considered later. The conclusion was at once evident that the mosaic fly had received from its father two yellow-bearing chromosomes, inseparable from one another, and that these inseparable

FIG. 1. *a*, Mosaic female fly (wings cut off); head, thorax (grey wings) and legs, wild type color (heterozygous for black); eyes slightly Bar; balancers and abdomen yellow (with light bands of yellow type). *b*, Triploid-X female, "grey" with abnormal wings, and flat, small abdomen.

chromosomes were transmitted together to the next generation, producing (wherever they occurred) females, because there were always two of them. No male offspring could be yellow, because no single yellow chromosome was transmitted (see Fig. 2). The sons must receive their X-chromosome from their father. The mosaic, as already stated, was not virgin; her brothers were all forked and some of them were bar, so that the two forked bar sons were in all probability offspring of a first mating and the other sons were offspring of the black male.

Three of the F_1 females were mated to three brothers, but no one of the pairs produced offspring; neither did a mass culture of F_1 males and females, nor did three F_1 males outcrossed to yellow-white females have any offspring. On the other hand, F_1 females outbred were fertile, and the sons of these were also fertile. The sterility of the F_1 males was expected if the X-chromosomes of the mosaic parent remained together, as the analysis shows, for then she should have produced two kinds of eggs (cf. Fig. 2), one kind with the double X and one kind with no X-chromosome. The XX-eggs fertilized by X-sperm would have produced XX X females (triploid-X females which would die); the XX-eggs, fertilized by Y-sperm, would have produced XXY females, the yellow daughters. The no-X or O-eggs, if fertilized by X-sperm, would have produced XO males, in appearance like the father, but sterile because lacking a Y-chromosome, and the O-eggs fertilized by Y-sperm would have produced YO males which would have died. Half the F_1 males and half the F_1 females presumably died and the sex-ratio remained 1:1. The actual numbers were 43 females to 59 males. The daughters were all yellow, but differed from their yellow mother in having, besides the "yellow-bearing" double chromosome, a Y-chromosome from their father; their sons therefore received from them a Y-chromosome and were fertile, and the surviving half, receiving their X-chromosome from the father, resembled him. Such patroclinous sons were produced by mating F_1 yellow daughters to wild type, to yellow white, to Xple, and to yellow broad, eosin, ruby males. The ratio of females to males in 8 cultures was 435:437, as would be expected again in this generation, since out of the four classes one class of females (XX X) would die, one class (XX Y) would survive, one class of

males (XY) would survive, and one class (YY) die (Fig. 2). In subsequent generations the line of double yellow females has remained intact; crosses have been made to many more types of males, always producing males like the father. The double yellow females are now used when it is desired to keep a supply of a certain type of male, for, since the X-chromosomes are inseparable, any egg which receives them produces a female, and only those eggs which receive the father's X can produce males. In such a line, then, there is never any crossing over in the X-chromosomes, and any desired combination of sex-linked characters can be maintained in the male flies by mating a male having the characters to a double yellow female. This is useful in cases where females of the race are weak or sterile, or where the pure cultures of the desired characters are weak, as frequently happens with a complicated combination of characters. The double yellow females are very vigorous and afford a reliable medium upon which to rear a race of feeble males. A possibility of a break in the continuity of a pure culture, which is easily discovered and rectified, will be discussed later.

The double yellow females probably behave like other flies in regard to the inheritance of characters in the autosomes. The original mosaic was dichete and transmitted the character normally. Crossing over in the second chromosome was tested by mating double yellow females to black purple vestigial lobe males and was found to be normal. Crossing over in the third chromosome was tested by mating double yellow females to pink, spineless, kidney, sooty, rough males, and found to be normal at the left end of the chromosome, but not at the right end; the discrepancy may, however, be due to the difficulty in classifying sooty in the presence of yellow.

X-Triploids.

After many hundreds of flies had been examined, there appeared occasionally, in cultures where the males were gray, wild type females of a more or less abnormal appearance; the eyes were somewhat rough and smaller than normal, the wings more or less imperfect and sometimes serrated, the abdomen imperfect, and the flies were weak (Fig. 1, *b*). These are characteristics of females

triploid for X. It is clear that from time to time a three X female survives. She has gray wings and body color like the father, because two yellow genes are recessive to one wild type gene. In a culture where the father was bar, the X-triploids had eyes less bar than in a heterozygous female, yet showing the character bar; this is known to be the appearance of bar when carried in one chromosome in the presence of two other chromosomes, both carrying the gene for wild type. X-triploids have not been observed in all of the cultures, and the percentage of them when present varies very much. In one culture their number was for a time about equal to the number of yellow females. All of many attempts to breed them have failed.

Occasional Breaking Apart of the Double Chromosome.

No exception to the non-criss-cross inheritance was observed until the F_4 generation, when a single yellow male appeared in a line in which all the other males were wild type. The yellow chromosomes of the mother had in this case presumably broken apart and the usual type of sex-linked inheritance from mother to son had been restored. The fly bred like an ordinary yellow male. At about the same time, in each of two related cultures of double yellow females by cross-veinless, cut, forked males, there appeared a wild type female not having the characteristics of an X-triploid. Both of these flies were fertile and both bred like females having one yellow-bearing X-chromosome, and one X-chromosome bearing the characters of the males of the cultures—*i.e.*, having the differentials for cross-veinless, cut and forked. One of these females was bred to a brother and produced females of two classes (wild type and cross-veinless, cut, forked) and yellow sons, and cross-veinless, cut, forked sons and the cross-over classes to be expected. The other wild type female was bred to a 7ple male and also produced yellow sons and cross-veinless, cut, forked sons, and females and cross-overs of the classes to be expected. These two females, like the yellow male of the other culture, had received from the mother a single yellow chromosome, broken apart from its mate. Since these flies occurred there have appeared in cultures of double yellow females six other yellow males and two wild type, not X-triploid, females. All the males have been bred and all but one

have produced offspring of the expected classes. Another female of the same kind undoubtedly occurred in a mass culture of double yellow females, for there were found in the culture some yellow

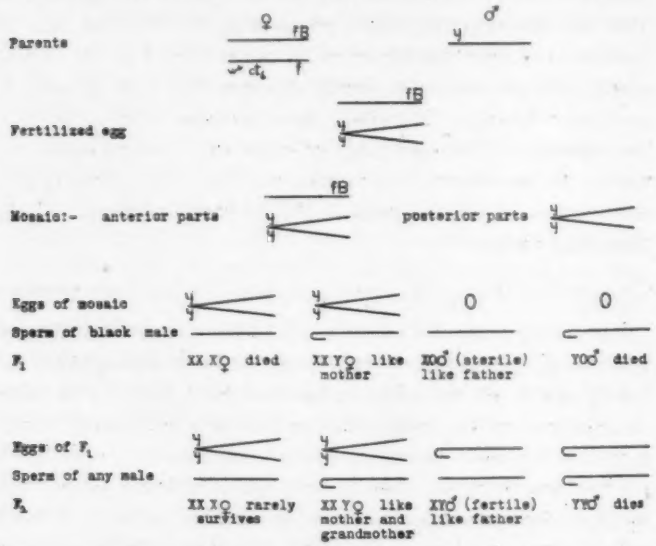

FIG. 2. Diagram showing parentage and constitution of the mosaic and of the line of non-criss-cross inheritance. The components of the double chromosome are arbitrarily represented as attached at "left" end.

males and cross-over males and females of classes to be expected if a female heterozygous for yellow had been present in the culture.

The question as to whether the break occurs in the place of the original attachment of the chromosomes has not been fully tested, but so far there is no evidence of duplication or deficiency at either end of the detached chromosome.

CYTOLOGY.

The genetic behavior of the line of flies having the two inseparable sex-chromosomes is in entire accord with the condition of the chromosomes as seen in cytological preparations of yellow females and of their rare wild type XX X sisters. Both of these classes of females receive from the mother not a single X, but two

X's attached to one another; the yellow female should have, in addition, a Y-chromosome from her father (Fig. 2, last line), the wild type female an additional X-chromosome from her father. Such chromosomes are, in fact, found in the maturation stages of the eggs of these females (Fig. 3, *b*, *c*, *d* and *e*, *f*, *g*, *h*). In both

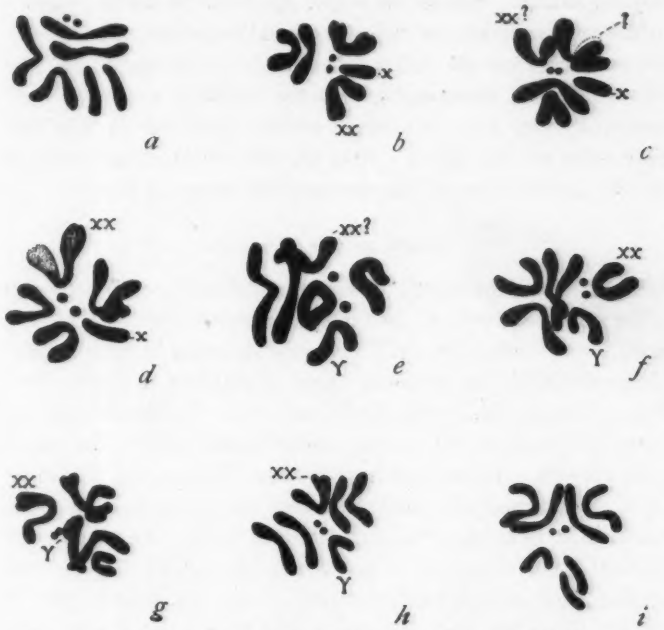

FIG. 3. Oögonial plates of: *a*, wild type female (after Bridges); *b*, *c*, *d*, triploid-X grey females (described in the text); *e*, *f*, *g*, *h*, double yellow females (described in text); *i*, non-disjunctional female of another origin (after Bridges), having three sex-chromosomes, shown for comparison. In all the figures are three pairs of autosomes. In the wild female (*a*) are two rod-shaped X-chromosomes (one from the father and one from the mother). In the triploid-X (*b*, *c*, *d*) and the double yellow (*e*, *f*, *g*, *h*) females is a single V-shaped body, the two attached X-chromosomes from the mother (sometimes with difficulty distinguishable from an autosome). In addition to these, there is: in the triploid-X females (*b*, *c*, *d*), a *rod-shaped* typical X-chromosome (derived from the father and accounting for the grey color of the fly); in the yellow females (*e*, *f*, *g*, *h*), the typical Y-chromosome (from the father). In the non-disjunctional female previously described by Bridges (*i*), the three sex-chromosomes are a Y-chromosome and two X-chromosomes not attached by their ends.

types there are the usual three pairs of autosomes, but the sex-chromosomes differ from the usual condition in that there is present in both types a U- or blunt V-shaped chromosome in place of a rod-shaped X-chromosome (Fig. 3, cf. *a* with *b, c, d* and *e, f, g, h*); this body is undoubtedly the united X's derived from the original mosaic. Besides the united X's from the mother, there is in the yellow female the typical hooked Y-chromosome, the sex-chromosome from the father (Fig. 3, *e, f, g, h*), and in the wild type female the chromosome from the father is the typical rod-shaped X (Fig. 3, *b, c, d*), whose presence gives her the wild type body color of her father. Thus the cytological evidence brings another confirmation of the chromosome theory of heredity.

Origin of the Mosaic.

The origin of the mosaic fly can be explained if at some division in the spermatogenesis of the father (perhaps at the equation division) the two halves of the X-chromosome failed to become completely detached, but remained fastened together at one of their ends, producing the V-shaped chromosome or double chromosome found in the germ cells of the female descendants. The sperm containing this chromosome must have fertilized an egg containing an X-chromosome with the differentials for forked and bar (this is known to have been the constitution of half of the eggs of the mother). An X-triploid fly was thus produced (cf. Fig. 2); the triploid condition remained, however, in only the anterior part of the fly, where the body color was wild type and the eyes were slightly bar. These characters, as stated, are as expected in an X-triploid, where two differentials for yellow are present with one for wild type, and one differential for bar with two for wild type. At an early stage the chromosome carrying forked and bar was eliminated, leaving part of the fly (the abdomen) with only two X-chromosomes, the attached yellow-bearing chromosomes (Fig. 2). The germ cells of the individual were derived from this part, and at the reduction division of the eggs there must have been extruded into the polar body (or remained in the egg) either the two attached X-chromosomes or no chromosome.

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